INHIBITION OF GLUCONEOGENESIS IN THE KIDNEYS BY A CYTOPLASMIC REGULATOR OF MITOCHONDRIAL MEMBRANE PERMEABILITY

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The writers showed previously that the cytoplasm of rat liver cells contains a thermostable glycoprotein which, in very low concentrations, inhibits mitochondrial membrane permeability for oxidation substrates [5]. Injection of insulin into rats increases the activity of this glycoprotein in the liver cytoplasm [4] and for that reason it was called insulin-dependent cytoplasmic regulator (IDR) [4]. On injection into intact rats IDR has a weak hypoglycemic action, but when injected into rats with hyperglycemia induced by alloxan it has a powerful hypoglycemic action [1]. Besides inhibition of glucose utilization by peripheral organs, another cause of the hyperglycemia in alloxan diabetes is known to be stimulation of gluconeogenesis in the liver and renal cortex [2, 6]. Inhibition of the outflow of glucose from pieces of liver on the addition of IDR was demonstrated previously [1].

The object of this investigation was to study whether inhibition of gluconeogenesis in the renal cortex is one possible cause of the hypoglycemic action of IDR.

EXPERIMENTAL METHOD

Pieces of kidney were obtained by forcing the renal cortex from rats deprived of food for 16 h through a press with holes 2 mm in diameter. Preincubation was carried out at 24°C in Krebs–Ringer bicarbonate buffer with different concentrations of Ca^{++}. Before free incubation, the incubation medium was saturated with oxygen and the experiment was carried out in flasks with tightly fitting ground glass stoppers, with intensive shaking. Glucose was measured by a modified orthotoluidine method [1]. Samples of 0.9 ml were taken and fixed by the addition of 0.1 ml 40% TCA. IDR was isolated from rat liver as described previously [4], and then desalinified on a column with Sephadex G-10.

EXPERIMENTAL RESULTS

As Fig. 1 shows, on the addition of IDR inhibition of gluconeogenesis was observed in the renal cortex with all substrates of gluconeogenesis used (pyruvate, lactate, succinate, α-ketoglutarate, glutamate). The most powerful inhibitory action of IDR was observed in the presence of pyruvate and, in particular, of lactate as substrate for gluconeogenesis. The weakest inhibition of gluconeogenesis on addition of IDR was observed when glutamate was used as the substrate.

It was suggested previously [11] that gluconeogenesis in the renal cortex may be limited by transport of substrates from the cytosol into the mitochondria. If this is so, inhibition of gluconeogenesis by IDR can be explained by its inhibitory action on activity of metabolite carriers in the mitochondrial membrane [5]. The first stage of gluconeogenesis in the renal cortex from pyruvate, α-ketoglutarate, succinate, and glutamate is the transport of these substrates from cytosol into mitochondria by carriers of monocarboxylates, α-ketoglutarate, dicarboxylic acids, and glutamate, respectively [3]. When lactate is used, it is transformed in the cytoplasm into pyruvate and, consequently, the functioning of a carrier of monocarboxylic acids in the mitochondrial membrane is also necessary. It is well known that activity of the monocarboxylic acid carrier in the mitochondrial membrane is much lower than

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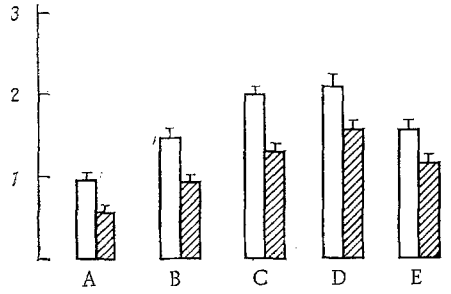

Fig. 1

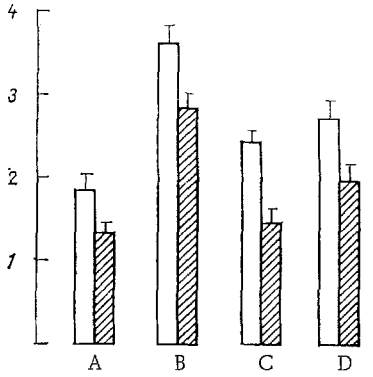

Fig. 2

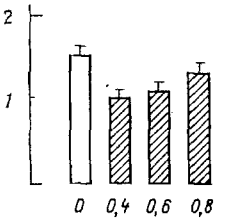

Fig. 3

Fig. 1. Inhibition of gluconeogenesis in pieces of renal cortex on addition of IDR. Substrate concentration 10 mM. Ca^{++} concentration in Krebs–Ringer bicarbonate buffer 0.2 mM, pH 7.4. A) Lactate as substrate; B) pyruvate, C) succinate, D) α-ketoglutarate, E) glutamate. Ordinate, gluconeogenesis (in mg glucose/g tissue/h). Unshaded columns) control, shaded) IDR (0.4 μg/ml.

Fig. 2. Inhibition of gluconeogenesis by IDR as a function of centration of Ca^{++} and H^{+} ions in incubation medium. Substrate 10 mM pyruvate. A) pH of medium 7.4; $CaCl_2$ concentration 0.2 mM; B) pH of medium 7.4; concentration of $CaCl_2$ 2.0 mM; C) pH of medium 7.6; $CaCl_2$ concentration 0.4 mM; D) pH of medium 6.9; $CaCl_2$ concentration 0.4 mM. Remainder of legend as to Fig. 1.

Fig. 3. Inhibition of gluconeogenesis as a function of IDR concentration. Substrate 10 mM pyruvate, pH 7.4; $CaCl_2$ concentration 0.2 mM. Abscissa, concentration of IDR (in μg/ml). Remainder of legend as to Fig. 1.

the activity of other substrate carriers [7]. Consequently, transport of pyruvate from cytosol into mitochondria ought to limit gluconeogenesis by a greater degree than the transport of other substrates of this process. Correspondingly, inhibition of pyruvate transport by IDR into the mitochondria ought to have a more serious effect on the intensity of gluconeogenesis from lactate or pyruvate than inhibition of carriers of dicarboxylic acids, α-ketoglutarate, and glutamate would have on the rate of gluconeogenesis from these substrates.

Hormonal control over the intensity of gluconeogenesis in the renal cortex is known to be largely effected through a change in the concentration of ionized Ca^{++} in the cytoplasm [10]. Since IDR increases the ability of mitochondria to accumulate Ca^{++} [4], one of the possible mechanisms of the action of IDR on gluconeogenesis in the renal cortex must be intensification of Ca^{++} accumulation from cytosol into mitochondria and, as a result, inhibition of gluconeogenesis because of a fall in the Ca^{++} concentration in the cytoplasm.

To test this hypothesis, dependence of the inhibitory action of IDR on gluconeogenesis on the Ca^{++} concentration in the incubation medium was studied. One of the distinguishing features of Ca^{++} transport in the kidney, compared with the liver, is that the intracellular concentration of Ca^{++} depends on its concentration in the external medium [8], evidently because of the participation of the kidney in transcellular Ca^{++} transport. It is known, [9, 11] that an increase in the Ca^{++} concentration in the incubation medium leads to activation of gluconeogenesis. It follows from Fig. 2 that similar results were obtained in the present investigation also: An increase in the Ca^{++} concentration from 0.2 to 2 mM led to stimulation of gluconeogenesis when pyruvate and succinate were used as the substrates. Inhibition of gluconeogenesis in this case was exhibited more strongly when Ca^{++} was present in a concentration of 0.2 mM than in a concentration of 2.0 mM. It is interesting to note that inhibition of gluconeogenesis when IDR was used was strengthened if the extracellular Ca^{++} concentration was increased from 0.2 to 0.4 mM but weakened with an increase in the Ca^{++} concentration to 2 mM (Fig. 2). No increase in the inhibitory action of IDR on gluconeogenesis could be observed when succinate was used on a change from 0.2 to 0.4 mM $CaCl_2$. As Fig. 2 shows, with an increase in pH of the incubation medium the inhibitory action of IDR on gluconeogenesis was intensified when pyruvate was used, whereas the opposite effect was observed when succinate was used.

The differences discovered in the relationship between the action of IDR on gluconeogenesis and pH and Ca^{++} concentration when the above-mentioned substances are used as substrates are evidently connected with differences in the mechanisms of transport of these substrates of gluconeogenesis from cytosol into mitochondria.

The decrease in sensitivity to IDR in high concentrations of Ca^{++} may be interpreted as an increase in the rate of inactivation of IDR in cells of the renal cortex and also of activation of the transport of substrates from cytosol into mitochondria by Ca^{++} [11]. Dependence of inhibition of gluconeogenesis when pyruvate is used on the concentration of IDR is illustrated in Fig. 3. A similar concentration dependence of the action of IDR was described by the writers previously in the case of inhibition of substrate transport into the mitochondria [5] and also for inhibition of the outflow of glucose from liver cells [2].

The results given in this paper thus confirm the view that the hypoglycemic action of IDR is partially attributable to inhibition of gluconeogenesis in the renal cortex. The existence of strong correlation between inhibition of gluconeogenesis and the block to the permeability of the mitochondrial membrane for substrates of gluconeogenesis discovered previously [5] indicates that inhibition of transport of metabolites from cytosol into mitochondria is the cause of inhibition of gluconeogenesis by IDR. It is unlikely that the action of IDR on gluconeogenesis is mediated through changes in Ca^{++} in the cytosol, for IDR does not prevent stimulation of gluconeogenesis by Ca^{++} ions.

Since the action of insulin on gluconeogenesis in the renal cortex is indirect in character [2], the possibility cannot be ruled out that besides ketone bodies, certain other factors transmit the action of insulin to the kidney. One such factor may perhaps be IDR, which is synthesized in the liver in response to injection of insulin [4].

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